

IAP12 Rec'd PCT/PTO 09 JUN 2006

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SEQUENCE LISTING

<110> University of Wales, Bangor

Trwyn Ltd

<120> Improvements In and Relating to Biosensors

<130> BA/SLH/Y1861

<160> 9

<170> PatentIn version 3.1

<210> 1

<211> 654

<212> DNA

<213> Escherichia coli K12

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accaactccc agccgtggca ttttattgtt gccagcacgg aagaaggtaa agcgcgtgtt	180
gccaaatccg ctgccccgtaa ttacgtgttc aacgagcgta aaatgcttga tgccctcgac	240
gtcgtggtgt tctgtcaaa aaccgcgtat gacgatgtct ggctgaagct ggttggatgtac	300
caggaagatg ccgtatggccg ctttgccacg ccggaaagcga aagccgcgaa cgataaaaggt	360
cgcaagttct tcgctgatat gcaccgtaaa gatctgcgtatg atgatgcaga gtggatggca	420
aaacaggttt atctcaacgt cggttaacttc ctgctcgccg tggccggctct gggctggac	480
gcggtagccca tcgaagggttt tgacgcccgc atcctcgatg cagaatttgg tctgaaagag	540
aaaggctaca ccagtctgggt ggttggcccg gtaggtcatc acagcggtga agattttaac	600
gctacgctgc cgaaatctcg tctgcccggaa aacatcacct taaccgaagt gtaa	654

<210> 2

<211> 826

<212> DNA

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<213> *Pseudomonas putida* JLR11

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agcgatcttc ctgtggatga gcagatgctg agctgggaga tcgcggcggc ccagtcagcc 180
tcgacttcct cgaacctgca agcttggagc gtgctcgccg tgcgggatcg cgagcgtctc 240
gcgaggcttg cccgactgtc cggttaaccag cgccatgtcg agcaggcacc gctgttcctg 300
gtctggctcg tggactggtc acgcctacgc cgactagcca gaacccttca ggcaccgact 360
gcaggtatcg actatttaga aagctacacc gtcggtgttg tagatgcagc tctggccgct 420
cagaacgccc cactagctt cgaggccaa ggactggaa tcgtttacat cggcgaaatg 480
cgcaaccacc cgaaagcgat gtccgaggag cttggcctgc caaacgacac tttcgctgta 540
tttggcatgt gcgtcggtca tcccgtatccg gcacagcccc ccgagatcaa gccacgcctg 600
gcgc当地 ctag tgggtgttca ccgtgagcgc tatgaggcca ccgaggcaga ggccgtttca 660
gttgctgcct atgaccgaag gatgagcgcac ttccaaacatc gtcaacaacg cgaaaaccgt 720
tcctggtcca gccaggccgt ggaacgtgta aaaggagcgg attcactgag cgaaagacac 780
cgcttgcgag atgcattaaa caccctaggt ttcggcctgc gctgag 826

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<212> DNA

<213> *Escherichia coli* K12 nfnB in pET-28(a)(+); pMKS2

<220>

<221> CDS

<222> (88)..(858)

<223> Coding sequence for nfnB gene

<220>

<221> misc_feature

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<222> (250)..(267)

<223> Cys tags

<220>

<221> misc_feature

<222> (160)..(177)

<223> His tags

<220>

<221> misc_feature

<222> (268)..(285)

<223> primer

<220>

<221> misc_feature

<222> (996)..(1010)

<223> primer

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tgtttaactt taagaaggag atataacc atg ggc agc agc cat cat cat cat cat 114
Met Gly Ser Ser His His His His His His
1 5cac agc agc ggc ctg gtg ccg cgc ggc agc cat atg gct agc atg act 162
His Ser Ser Gly Leu Val Pro Arg Gly Ser His Met Ala Ser Met Thr
10 15 20 25gg t gga cag caa atg ggt cgc gga tcc tgt tgc tgt tgc tgc gat 210
Gly Gly Gln Met Gly Arg Gly Ser Cys Cys Cys Cys Cys Asp
30 35 40atc att tct gtc gcc tta aag cgt cat tcc act aag gca ttt gat gcc 258
Ile Ile Ser Val Ala Leu Lys Arg His Ser Thr Lys Ala Phe Asp Ala
45 50 55agc aaa aaa ctt acc ccg gaa cag gcc gag cag atc aaa acg cta ctg 306
Ser Lys Lys Leu Thr Pro Glu Gln Ala Glu Gln Ile Lys Thr Leu Leu
60 65 70

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caa tac agc cca tcc agc acc aac tcc cag ccg tgg cat ttt att gtt		354	
Gln Tyr Ser Pro Ser Ser Thr Asn Ser Gln Pro Trp His Phe Ile Val			
75	80	85	
gcc agc acg gaa gaa ggt aaa gcg cgt gtt gcc aaa tcc gct gcc ggt		402	
Ala Ser Thr Glu Glu Gly Lys Ala Arg Val Ala Lys Ser Ala Ala Gly			
90	95	100	105
aat tac gtg ttc aac gag cgt aaa atg ctt gat gcc tcg cac gtc gtg		450	
Asn Tyr Val Phe Asn Glu Arg Lys Met Leu Asp Ala Ser His Val Val			
110	115	120	
gtg ttc tgt gca aaa acc gcg atg gac gat gtc tgg ctg aag ctg gtt		498	
Val Phe Cys Ala Lys Thr Ala Met Asp Asp Val Trp Leu Lys Leu Val			
125	130	135	
gtt gac cag gaa gat gcc gat ggc cgc ttt gcc acg ccg gaa gcg aaa		546	
Val Asp Gln Glu Asp Ala Asp Gly Arg Phe Ala Thr Pro Glu Ala Lys			
140	145	150	
gcc gcg aac gat aaa ggt cgc aag ttc ttc gct gat atg cac cgt aaa		594	
Ala Ala Asn Asp Lys Gly Arg Lys Phe Phe Ala Asp Met His Arg Lys			
155	160	165	
gat ctg cat gat gat gca gag tgg atg gca aaa cag gtt tat ctc aac		642	
Asp Leu His Asp Asp Ala Glu Trp Met Ala Lys Gln Val Tyr Leu Asn			
170	175	180	185
gtc ggt aac ttc ctg ctc ggc gtg gcg gct ctg ggt ctg gac gcg gta		690	
Val Gly Asn Phe Leu Leu Gly Val Ala Ala Leu Gly Leu Asp Ala Val			
190	195	200	
ccc atc gaa ggt ttt gac gcc gcc atc ctc gat gca gaa ttt ggt ctg		738	
Pro Ile Glu Gly Phe Asp Ala Ala Ile Leu Asp Ala Glu Phe Gly Leu			
205	210	215	
aaa gag aaa ggc tac acc agt ctg gtg gtt gtt ccg gta ggt cat cac		786	
Lys Glu Lys Gly Tyr Thr Ser Leu Val Val Val Pro Val Gly His His			
220	225	230	
agc gtt gaa gat ttt aac gct acg ctg ccg aaa tct cgt ctg ccg caa		834	
Ser Val Glu Asp Phe Asn Ala Thr Leu Pro Lys Ser Arg Leu Pro Gln			
235	240	245	
aac atc acc tta acc gaa gtg taa ttctctcttg ccggcatct gccccgtat		888	
Asn Ile Thr Leu Thr Glu Val			
250	255		
ttcctctcag attctcctga tttgcataac cctgtttcag caagttcgt catcataggc		948	
tgctgttcaa gcttgccgc gcactcgagc accaccacca ccaccactga gatccggctg		1008	
ctaacaaagc ccgaaaggaa gctgagttgg ctgctgccac cgctgagcaa taactagc		1066	

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<212> PRT

<213> Escherichia coli K12 nfnB in pET-28(a) (+); pMKS2

<220>

<221> misc_feature

<222> (250)..(267)

<223> Cys tags

<220>

<221> misc_feature

<222> (160)..(177)

<223> His tags

<220>

<221> misc_feature

<222> (268)..(285)

<223> primer

<220>

<221> misc_feature

<222> (996)..(1010)

<223> primer

<400> 4

Met Gly Ser Ser His His His His His Ser Ser Gly Leu Val Pro
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Arg Gly Ser His Met Ala Ser Met Thr Gly Gly Gln Gln Met Gly Arg
20 25 30

Gly Ser Cys Cys Cys Cys Cys Asp Ile Ile Ser Val Ala Leu Lys
35 40 45

Arg His Ser Thr Lys Ala Phe Asp Ala Ser Lys Lys Leu Thr Pro Glu
50 55 60

Gln Ala Glu Gln Ile Lys Thr Leu Leu Gln Tyr Ser Pro Ser Ser Thr
65 70 75 80

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Asn Ser Gln Pro Trp His Phe Ile Val Ala Ser Thr Glu Glu Gly Lys
85 90 95

Ala Arg Val Ala Lys Ser Ala Ala Gly Asn Tyr Val Phe Asn Glu Arg
100 105 110

Lys Met Leu Asp Ala Ser His Val Val Val Phe Cys Ala Lys Thr Ala
115 120 125

Met Asp Asp Val Trp Leu Lys Leu Val Val Asp Gln Glu Asp Ala Asp
130 135 140

Gly Arg Phe Ala Thr Pro Glu Ala Lys Ala Ala Asn Asp Lys Gly Arg
145 150 155 160

Lys Phe Phe Ala Asp Met His Arg Lys Asp Leu His Asp Asp Ala Glu
165 170 175

Trp Met Ala Lys Gln Val Tyr Leu Asn Val Gly Asn Phe Leu Leu Gly
180 185 190

Val Ala Ala Leu Gly Leu Asp Ala Val Pro Ile Glu Gly Phe Asp Ala
195 200 205

Ala Ile Leu Asp Ala Glu Phe Gly Leu Lys Glu Lys Gly Tyr Thr Ser
210 215 220

Leu Val Val Val Pro Val Gly His His Ser Val Glu Asp Phe Asn Ala
225 230 235 240

Thr Leu Pro Lys Ser Arg Leu Pro Gln Asn Ile Thr Leu Thr Glu Val
245 250 255

<210> 5

<211> 1221

<212> DNA

<213> *Pseudomonas putida* JLR11 prnB in pET-28(a) (+) ; pKMS6

<220>

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<221> CDS

<222> (88)..(1029)

<223>

<220>

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<222> (190)..(225)

<223> primer

<220>

<221> misc_feature

<222> (190)..(207)

<223> cys tag

<220>

<221> misc_feature

<222> (936)..(956)

<223> primer

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tgtttaactt taagaaggag atatacc atg ggc agc agc cat cat cat cat 114
Met Gly Ser Ser His His His His His His
1 5cac agc agc ggc ctg gtg ccg cgc ggc agc cat atg gct agc atg act 162
His Ser Ser Gly Leu Val Pro Arg Gly Ser His Met Ala Ser Met Thr
10 15 20 25ggt gga cag caa atg ggt cgc gga tcc tgt tgc tgt tgc tgt tgc agc 210
Gly Gly Gln Gln Met Gly Arg Gly Ser Cys Cys Cys Cys Cys Cys Ser
30 35 40ctt caa gac gaa gca ctc aaa gcc tgg caa gcc cgt tat ggc gag cca 258
Leu Gln Asp Glu Ala Leu Lys Ala Trp Gln Ala Arg Tyr Gly Glu Pro
45 50 55gct aac tta cct gct gcc gac acc gtg atc gcg cag atg ttg cag cat 306
Ala Asn Leu Pro Ala Ala Asp Thr Val Ile Ala Gln Met Leu Gln His

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60

65

70

cga tca gta cgt gcc tac agc gat ctt cct gtg gat gag cag atg ctg			354
Arg Ser Val Arg Ala Tyr Ser Asp Leu Pro Val Asp Glu Gln Met Leu			
75	80	85	
agc tgg gcg atc gcg gcg gcc cag tca gcc tcg act tcc tcg aac ctg			402
Ser Trp Ala Ile Ala Ala Gln Ser Ala Ser Thr Ser Ser Asn Leu			
90	95	100	105
caa gct tgg agc gtg ctc gcc gtg cggt gat cgc gag cgt ctc gcg agg			450
Gln Ala Trp Ser Val Leu Ala Val Arg Asp Arg Glu Arg Leu Ala Arg			
110	115	120	
ctt gcc cga ctg tcc ggt aac cag cgc cat gtc gag cag gca ccg ctg			498
Leu Ala Arg Leu Ser Gly Asn Gln Arg His Val Glu Gln Ala Pro Leu			
125	130	135	
ttc ctg gtc tgg ctc gtg gac tgg tca cgc cta cgc cga cta gcc aga			546
Phe Leu Val Trp Leu Val Asp Trp Ser Arg Leu Arg Arg Leu Ala Arg			
140	145	150	
acc ctt cag gca ccg act gca ggt atc gac tat tta gaa agc tac acc			594
Thr Leu Gln Ala Pro Thr Ala Gly Ile Asp Tyr Leu Glu Ser Tyr Thr			
155	160	165	
gtc ggt gtt gta gat gca gct ctg gcc gct cag aac gcc gca cta gct			642
Val Gly Val Val Asp Ala Ala Leu Ala Ala Gln Asn Ala Ala Leu Ala			
170	175	180	185
ttc gag gcc caa gga ctg gga atc gtt tac atc ggc gga atg cgc aac			690
Phe Glu Ala Gln Gly Leu Gly Ile Val Tyr Ile Gly Gly Met Arg Asn			
190	195	200	
cac ccg gaa gcg atg tcc gag gag ctt ggc ctg cca aac gac act ttc			738
His Pro Glu Ala Met Ser Glu Glu Leu Gly Leu Pro Asn Asp Thr Phe			
205	210	215	
gct gta ttt ggc atg tgc gtc ggt cat ccc gat ccg gca cag ccc gcc			786
Ala Val Phe Gly Met Cys Val Gly His Pro Asp Pro Ala Gln Pro Ala			
220	225	230	
gag atc aag cca cgc ctg gcg caa tca gtg gtg ctt cac cgt gag cgc			834
Glu Ile Lys Pro Arg Leu Ala Gln Ser Val Val Leu His Arg Glu Arg			
235	240	245	
tat gag gcc acc gag gca gag gcg gtt tca gtt gct gcc tat gac cga			882
Tyr Glu Ala Thr Glu Ala Glu Ala Val Ser Val Ala Ala Tyr Asp Arg			
250	255	260	265
agg atg agc gac ttc caa cat cgt caa cca cgc gaa aac cgt tcc tgg			930
Arg Met Ser Asp Phe Gln His Arg Gln Gln Arg Glu Asn Arg Ser Trp			
270	275	280	
tcc agc cag gcc gtg gaa cgt gta aaa gga gcg gat tca ctg agc gga			978
Ser Ser Gln Ala Val Glu Arg Val Lys Gly Ala Asp Ser Leu Ser Gly			
285	290	295	
aga cac cgc ttg cga gat gca tta aac acc cta ggt ttc ggc ctg cgc			1026

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Arg His Arg Leu Arg Asp Ala Leu Asn Thr Leu Gly Phe Gly Leu Arg		
300	305	310
tga gatagtgaga tatcccatgc ctattccgc cgccctgaac cggagcacta		1079
ataacctggca actttgcttgc agctccgtcg acaagcttgc ggccgcactc gagcaccacc		1139
accaccacca ctgagatccg gctgctaaca aagcccgaaa ggaagctgag ttggctgctg		1199
ccaccgctga gcaataacta gc		1221

<210> 6

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<212> PRT

<213> *Pseudomonas putida* JLR11 prnB in pET-28(a) (+) ; pKMS6

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<221> misc_feature

<222> (190)..(225)

<223> primer

<220>

<221> misc_feature

<222> (190)..(207)

<223> cys tag

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<221> misc_feature

<222> (936)..(956)

<223> primer

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Met Gly Ser Ser His His His His His Ser Ser Gly Leu Val Pro			
1	5	10	15

Arg Gly Ser His Met Ala Ser Met Thr Gly Gly Gln Gln Met Gly Arg		
20	25	30

Gly Ser Cys Cys Cys Cys Cys Ser Leu Gln Asp Glu Ala Leu Lys		
35	40	45

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Ala Trp Gln Ala Arg Tyr Gly Glu Pro Ala Asn Leu Pro Ala Ala Asp
50 55 60

Thr Val Ile Ala Gln Met Leu Gln His Arg Ser Val Arg Ala Tyr Ser
65 70 75 80

Asp Leu Pro Val Asp Glu Gln Met Leu Ser Trp Ala Ile Ala Ala Ala
85 90 95

Gln Ser Ala Ser Thr Ser Ser Asn Leu Gln Ala Trp Ser Val Leu Ala
100 105 110

Val Arg Asp Arg Glu Arg Leu Ala Arg Leu Ala Arg Leu Ser Gly Asn
115 120 125

Gln Arg His Val Glu Gln Ala Pro Leu Phe Leu Val Trp Leu Val Asp
130 135 140

Trp Ser Arg Leu Arg Arg Leu Ala Arg Thr Leu Gln Ala Pro Thr Ala
145 150 155 160

Gly Ile Asp Tyr Leu Glu Ser Tyr Thr Val Gly Val Val Asp Ala Ala
165 170 175

Leu Ala Ala Gln Asn Ala Ala Leu Ala Phe Glu Ala Gln Gly Leu Gly
180 185 190

Ile Val Tyr Ile Gly Gly Met Arg Asn His Pro Glu Ala Met Ser Glu
195 200 205

Glu Leu Gly Leu Pro Asn Asp Thr Phe Ala Val Phe Gly Met Cys Val
210 215 220

Gly His Pro Asp Pro Ala Gln Pro Ala Glu Ile Lys Pro Arg Leu Ala
225 230 235 240

Gln Ser Val Val Leu His Arg Glu Arg Tyr Glu Ala Thr Glu Ala Glu
245 250 255

Ala Val Ser Val Ala Ala Tyr Asp Arg Arg Met Ser Asp Phe Gln His
260 265 270

Arg Gln Gln Arg Glu Asn Arg Ser Trp Ser Ser Gln Ala Val Glu Arg
275 280 285

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Val Lys Gly Ala Asp Ser Leu Ser Gly Arg His Arg Leu Arg Asp Ala
290 295 300

Leu Asn Thr Leu Gly Phe Gly Leu Arg
305 310

<210> 7

<211> 24

<212> DNA

<213> Escherichia coli

<400> 7

ggatccgata tcatttctgt cgcc

24

<210> 8

<211> 27

<212> DNA

<213> Escherichia coli

<400> 8

cgtcatcata ggctgctgtt gaagctt

27

<210> 9

<211> 42

<212> DNA

<213> Artificial Sequence

<220>

<223> Primer consisting of nfnB gene primer shown in SEQ ID4 with an additional 6 cysteine codons

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42